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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No	GI 5387	Total Pages	16
First Named Inventor or Application Identifier			
Hyun Kim			

JC954 U.S. PTO 10/13/00

JC841 U.S. PTO 09/688281 10/13/00

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Assistant Commissioner for Patents**
Box Patent Application
Washington, DC 20231

1. Fee Transmittal Form

(Submit an original, and a duplicate for fee processing)

2. Specification [Total Page]
X 16

(preferred arrangement set forth below)
- Descriptive title of the invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings *(if filed)*
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. Drawings *(35 USC 113)* [Total Sheets]
X - Informal 4

4. Oath or Declaration [Total Pages]
X 6

a. Signed (copy)

b. Copy from a prior application (37 CFR 1.63(d))

(for continuation/divisional with Box 17 completed)
[Note Box 5 below]

I. DELETION OF INVENTOR(S)

Signed statement attached deleting inventor(s) named in the prior application, See 37 CFR 1.63(d)(2) and 1.33(b).

5. Incorporation By Reference *(useable if Box 4b is checked)*

The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. Microfiche Computer Program *(Appendix)*

7. Nucleotide and/or Amino Acid Sequence Submission *(If applicable, all necessary)*

a. Computer Readable Copy

b. Paper Copy (identical to computer copy) (pp.)

ACCOMPANYING APPLICATION PARTS

8. Assignment Papers (cover sheet & documents(s))

9. 37 CFR 3.73(b) Statement Power of Attorney

(when there is an assignee)

10. English Translation Document *(if applicable)*

11. Information Disclosure Copies of IDS

Statement (IDS)/PTO-1449 Citations

12. Preliminary Amendment [Pages]

13. Return Receipt Postcard (MPEP 503)
X

(Should be specifically itemized)

14. Small Entity Statement filed in prior application,

Statement(s) Status still proper and desired

15. Certified Copy of Priority Document(s)

(if foreign priority is claimed)

16. *Other:* . .

17. **If a CONTINUING APPLICATION,** *check appropriate box and supply the requisite information:*
Continuation of prior application No.:

18. CORRESPONDENCE ADDRESS					
Customer Number or Bar Code Label	(Insert Customer No. or Attach bar code label here)			or Correspondence address below	X
NAME	Barbara A. Gyure				
	American Home Products Corporation				
ADDRESS	Patent & Trademark Office - 2B (Attn: Kay E. Brady)				
	One Campus Drive				
CITY	Parsippany	STATE	NJ	ZIP CODE	07054
COUNTRY	U.S.A.	TELEPHONE	(617) 665-8653	FAX	(617) 876-5851

SUBMITTED BY					
Typed or Printed Name	Barbara A. Gyure		Reg. No.	34,614	
Signature	Barbara A. Gyure	Date	Oct. 13, 2000	Deposit Account	07-1060

CERTIFICATE OF EXPRESS MAILING

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Typed or printed name	Jill Marcotte		
Signature	Jill Marcotte	Date	10/13/00

Date: 10/13/00 Express Mail Label No. EL564796679US

Inventors: Hyun Kim, Rebecca Li, Alessandra Pavesio, Lanfranco Callegaro, Howard Seeherman and John Wozney

Attorney's Docket No.: GI 5387

INJECTABLE CARRIER FORMULATIONS OF HYALURONIC ACID DERIVATIVES FOR DELIVERY OF OSTEOGENIC PROTEINS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/159,674 filed on October 15, 1999 and U.S. Provisional Application No. 60/185,587 filed on February 28, 2000.

BACKGROUND OF THE INVENTION

The subject invention relates to the field of osteogenic proteins and pharmaceutical formulations thereof. More particularly, the subject invention involves injectable pharmaceutical formulations comprising hyaluronic acid derivatives and osteogenic proteins. The invention further provides methods for formulating porous injectable gels and pastes from hyaluronic acid.

Osteogenic proteins are those proteins capable of inducing, or assisting in the induction of, cartilage and/or bone formation. Many such osteogenic proteins have in recent years been isolated and characterized, and some have been produced by recombinant methods. For example, so-called bone morphogenic proteins (BMP) have been isolated from demineralized bone tissue (see e.g. Urist US 4,455,256); a number of such BMP proteins have been produced by recombinant techniques (see e.g. Wang et al. US 4,877,864 and Wang et al. US 5,013,549); a family of transforming growth factors (TGF-α and TGF-β) has been identified as potentially useful in the treatment of bone

disease (see e.g. Derynck et al., EP 154,434); a protein designated Vgr-1 has been found to be expressed at high levels in osteogenic cells (see Lyons et al. (1989) Proc. Nat'l. Acad. Sci. USA 86, 4554-4558); and proteins designated OP-1, COP-5 and COP-7 have purportedly shown bone inductive activity (see Oppermann, et al. U.S. 5,001,691).

Various formulations designed to deliver osteogenic proteins to a site where induction of bone formation is desired have been developed. For example, certain polymeric matrices such as acrylic ester polymer (Urist, US 4,526,909) and lactic acid polymer (Urist, US 4,563,489) have been utilized.

A biodegradable matrix of porous particles for delivery of an osteogenic protein designated as OP is disclosed in Kuber A. Sampath, U.S. 5,108,753.

Brekke et al., United States Patents 4,186,448 and 5,133,755 describe methods of forming highly porous biodegradable materials composed of polymers of lactic acid ("OPLA").

Okada et al., US 4,652,441, US 4,711,782, US 4,917,893 and US 5,061,492 and Yamamoto et al., US 4,954,298 disclose a prolonged-release microcapsule comprising a polypeptide drug and a drug-retaining substance encapsulated in an inner aqueous layer surrounded by a polymer wall substance in an outer oil layer.

Yamazaki et al., Clin. Orthop. and Related Research, 234:240-249 (1988) disclose the use of implants comprising 1 mg of bone morphogenetic protein purified from bone and 5 mg of Plaster of Paris. United States Patent 4,645,503 discloses composites of hydroxyapatite and Plaster of Paris as bone implant materials.

Collagen matrices have also been used as delivery vehicles for osteogenic proteins (see e.g. Jeffries, U.S. 4,394,370).

SUMMARY OF THE INVENTION

The present invention provides injectable formulations for delivery of osteogenic proteins. In one embodiment the composition comprises the osteogenic protein and hyaluronic acid esters. In another embodiment, the composition may further include tricalcium phosphate. The injectable formulations of the invention allows for closed

fracture repair and other skeletal tissue without an open reduction procedure as is necessary with implantable devices.

The present invention further provides methods for preparing injectable gels or pastes useful as a carrier for osteogenic proteins by transforming various non-woven pads and sponges of hyaluronic acid benzyl ester into injectable gel or paste formulations by hydration or solvent addition. In another embodiment, the invention comprises compositions comprising the transformed injectable gel or paste formulations.

The methods and compositions of the present invention are useful for the preparation of formulations of osteoinductive proteins which can be used, among other uses, to promote the formation of cartilage and/or bone, for repair of tissue damage and fractures. The invention further provides methods for treating patients in need of cartilage and/or bone repair and/or growth.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 sets forth in vitro release kinetics of ^{125}I-rhBMP-2 in Hyaff gels.

Figure 2 sets forth in vivo retention of ^{125}I-rhBMP-2 in Hyaff-11/PEG, ACS, and buffer.

Figure 3 sets forth in vitro release kinetics of ^{125}I-rhBMP-2 in Hyaff gels/TCP.

Figure 4 sets forth in vivo biodistribution of ^{125}I-rhBMP-2.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides injectable formulations for delivery of osteogenic proteins. The compositions comprise an injectable formulation of hyaluronic acid esters and osteogenic protein. The present invention further provides processes for preparing injectable gel or paste formulations by transforming various non-woven pads and sponges of hyaluronic acid benzyl ester by hydration or solvent addition yielding gels with in vivo residence times from days to up to several months. Total or partial esters of hyaluronic acid are described in US 5,336,767. Partial esters of Hyaff solids are transformed into gels using aqueous buffer or organic solvents (such as N-methyl

pyrrolidinone, dimethyl sulfoxide, etc), while complete esters of Hyaff solids are transformed into gels using organic solvents. In other embodiments pore formers may be introduced to the solublized carriers to increase porosity. The addition of pore formers would allow in situ pore formation after injection in vivo by solubilization of pore former and precipitation/phase inversion of carrier. Suitable liquid pore formers include polyethylene glycol or PEG at 10 - 90 % volume per volume ratios) and solid pore formers (such as sodium bicarbonate, sodium chloride, citric acid, sucrose, etc., at 1:1 - 21:1 pore former:Hyaff weight per weight ratios) to increase porosity. The gel/paste can also contain TCP (tri-calcium phosphate) particles as a mineral component for example, at 0.1 - 100 % weight per volume range.

The amount , type and size of the pore forming agent is optimized to leave voids sufficient for cell ingrowth into injectable gel when pore forming agent and solvent are extracted from the carrier in vivo by solubilization of pore forming agent and precipitation/phase inversion of carrier in situ.

The osteogenic proteins useful with the injectable carriers made in accordance with the subject invention are well known to those skilled in the art and include those discussed above. The preferred osteogenic proteins for use herein are those of the BMP class identified as BMP-1 through BMP-12 in US 4,877,864; US 5,013,649; WO 90/11366 published October 4, 1990; WO 91/18098 published November 28, 1991; WO 93/00432, published January 7, 1993; United States Serial Numbers 08/247,908 and 08/247,904, both filed May 20, 1994; and United States Serial Number 08/217,780, filed on March 25, 1994. The disclosure of the above publications are hereby incorporated by reference. The most preferred is BMP-2, the full length cDNA sequence of which is described in detail in the '649 patent. Of course, combinations of two or more of such osteogenic proteins may be used, as may fragments of such proteins that also exhibit osteogenic activity. Such osteogenic proteins are known to be homodimeric species, but also exhibit activity as mixed heterodimers. Heterodimeric forms of osteogenic proteins may also be used in the practice of the subject invention.

BMP heterodimers are described in WO93/09229, the disclosure of which is hereby incorporated by reference. Recombinant proteins are preferred over naturally occurring isolated proteins. The amount of osteogenic protein useful herein is that amount effective to stimulate increased osteogenic activity of infiltrating progenitor cells, and will depend upon the size and nature of defect being treated as well as the carrier being employed.

The formulations may be injected for example into tendons, damaged cartilage tissue, ligaments, and/or their attachment sites to bones. Injectable formulations may also find application to other bone sites such as bone cysts, bone defects, intraosseous sites and closed fractures.

The dosage regimen will be determined by the clinical indication being addressed, as well as by various patient variables (e.g. weight, age, sex) and clinical presentation (e.g. extent of injury, site of injury, etc.). In general, the dosage of osteogenic protein will be in the range of from about 0.1 to 4 mg/ml.

The injectable osteogenic protein formulations may be provided to the clinic as a single formulation, or the formulation may be provided as a multicomponent kit wherein, e.g. the osteogenic protein is provided in one vial and the injectable hyaluronic paste is provided separately.

The compositions of the subject invention allow therapeutically effective amounts of osteoinductive protein to be delivered to an injury site where cartilage and/or bone formation is desired. The formulations may be used as a substitute for autologous bone graft in fresh and non-union fractures, spinal fusions, and bone defect repair in the orthopaedic field; in cranio/maxillofacial reconstructions; for prosthesis integration, especially as a surface coating to improve fixation of prosthetic implants such as hydroxyapatite coated prostheses; in osteomyelitis for bone regeneration; and in the dental field for augmentation of the alveolar ridge and periodontal defects and tooth extraction sockets. The methods and formulations of the present invention may be useful in the treatment and/or prevention of osteoporosis, or the treatment of

osteoporotic or osteopenic bone. In another embodiment, formulations of the present invention may be used in the process known as distraction osteogenesis. When used to treat osteomyelitis or for bone repair with minimal infection, the osteogenic protein may be used in combination with porous microparticles and antibiotics, with the addition of protein sequestering agents such as alginate, cellulosics, especially carboxymethylcellulose, diluted using aqueous glycerol. The antibiotic is selected for its ability to decrease infection while having minimal adverse effects on bone formation. Preferred antibiotics for use in the devices of the present invention include vancomycin and gentamycin. The antibiotic may be in any pharmaceutically acceptable form, such as vancomycin HCl or gentamycin sulfate. The antibiotic is preferably present in a concentration of from about 0.1 mg/mL to about 10.0 mg/mL. The traditional preparation of formulations in pharmaceutically acceptable form (i.e. pyrogen free, appropriate pH and isotonicity, sterility, etc.) is well within the skill in the art and is applicable to the formulations of the invention.

Hyaluronic derivative compositions of the invention prepared by hydration or solvent addition of insoluble or partially soluble non- woven pads or sponges may also be ultilized in combination with other drugs, growth factors, peptides, proteins, cytokines, oligonucleotides antisense oligonucleotides, DNA and polymers. These compounds may be added by mixing them with the carriers. Or by covalent attachment to the polymer carriers. The hyaluronic derivative compositions may also be used with DNA encoding for BMPs and cells transduced or transfected with genes encoding BMP proteins.

The following examples are illustrative of the present invention and are not limiting in any manner. Modifications, variations and minor enhancements are contemplated and are within the present invention.

EXAMPLE 1

PREPARATION OF INJECTABLE HYALURONIC ACID ESTERS

The starting Hyaff hyaluronic acid (Fidia Advanced Biopolymers, Abano Terme, Italy) materials are solids such as non-woven pads, felts, sheets, powders, sponges, and microspheres. The Hyaff materials are esters of hyaluronic acid exhibiting various ester moities (e.g., benzyl, ethyl,propyl pentyl or larger molecules such as hydrocortisone or methyl prednislone, etc.) as well as various degrees of esterification (i.e., partial esters or complete esters). Partial esters of Hyaff are designated by percent esterfication ranging from 50-99 % (e.g., Hyaff-11p65, Hyaff-11p80, etc.), while complete esters are 100 % esters of hyaluronic acid (e.g., Hyaff-11).

Hyaff gel classification used in supporting data is as follows and is followed by examples of select formulations:

- Hyaff-11 gel: Hyaff-11 non-woven pad transformed into gel with organic solvent to yield 10 % solids
- Hyaff-11/bicarbonate gel: Hyaff-11 gel mixed with sodium bicarbonate as pore former at 15:1 (w/w) bicarbonate to Hyaff-11
- Hyaff-11/PEG gel: Hyaff-11 gel mixed with polyethylene glycol(200mw) as pore former at 33 - 50 % (v/v) range
- Hyaff-11/TCP gel: Hyaff-11 gel mixed with 30 % w/v TCP
- Hyaff-11/bicarbonate/TCP gel: Hyaff-11/bicarbonate gel mixed with 30 % w/v TCP
- Hyaff-11/PEG/TCP gel: Hyaff-11/PEG gel mixed with 30 % w/v TCP
- Hyaff-11p80 gel: Hyaff-11p80 non-woven pad transformed into gel with organic solvent to yield 5 % solids
- Hyaff-11p65 gel: Hyaff-11p65 non-woven pad hydrated with aqueous buffer to yield 6 - 15 % solids
- Hyaff-11p65/TCP gel: Hyaff-11p65 gel mixed with 30 % w/v TCP

Hyaff-11p65 non-woven pads were hydrated with glutamic acid buffer (pH 4.5) containing rhBMP-2 (0.1 mg/mL final conc.) to yield either 6 % - 15 % solids (w/v) and mixed thoroughly to form a paste. Hyaff-11p80 and Hyaff-11 non-woven pads were solubilized in N-methyl-pyrrolidinone (NMP) or dimethyl sulfoxide(DMSO)to yield a 1 - 30 % w/v solution. These solutions were then mixed with either rhBMP-2-containing buffer (10 % v/v, 0.1 mg/mL rhBMP-2), or lyophilized rhBMP-2 (0.1 mg/mL) followed by the addition of various pore formers (polyethylene glycol, sodium bicarbonate, sucrose, NaCl, citric acid)and tricalcium phosphate (TCP). Particle size of solid pore formers and TCP used was < 600um, preferably <200umLiquid pore formers such as PEG(200mw) were mixed at 10-90% v/v ratios, and solid pore formers were mixed at 9:1 - 21:1 (w/w) pore former to carrier ratios. TCP was mixed at 0.1-30% (w/v). TCP (45-125 micron particle size) was mixed thoroughly into rhBMP-2/Hyaff-11 or rhBMP-2/Hyaff-11p65 gel at 30% (w/v). Separately, rhBMP-2 was adsorbed onto TCP first, followed by mixing with Hyaff-11 or Hyaff-11p65 gel. Formulations were chosen based on injectability through an 18 g needle. Microstructure was characterized by scanning electron microscopy (SEM).

SEMS revealed varying degrees of pore structure and porosity. Hyaff-11p65 6% gel exhibited longer fibers than the 15% formulation; with both displaying a high level of porosity. Both Hyaff-11 and Hyaff-11p80 gels showed minimal pore structure and porosity, whereas those carriers with pore formers displayed a high level of porosity. Pore formers and/ or additives that yielded injectable mixtures were PEG, sodium bicarbonate and TCP.

EXAMPLE 2

IN VITRO RELEASE KINETICS

rhBMP-2 was radiolabeled with ^{125}I using the Iodogen method (Pierce) and used as a tracer for 0.1 mg/ml rhBMP-2 delivered in 100 ul Hyaff-11p65 gel, Hyaff-11p80 gel, Hyaff-11gel and Hyaff-11/PEG (n=4). ^{125}I-rhBMP-2 loaded samples (50,000

cpm/sample) were incubated in 1 ml fetal calf serum (Hyclone) at 37°C on a shaker, and radioactivity of the carrier measured up to 14 days using a gamma counter. Fresh serum was replaced after each time point. ^{125}I-rhBMP-2 release from injectable formulations were compared to those of implantable sponges and pads of Hyaff-11 and Hyaff-11p80.

Auto cross-linked polysaccharide form of derivatized hyaluronic acid, ACP gel, is used for the in vitro release study and the rat ectopic assay. For the in vitro release study, 2 ml ACP gel is mixed with 1.53 mg rhBMP-2 cake (which corresponds to 0.2 mg actual rhBMP-2 at 8 mg rhBMP-2 per 61 mg cake weight) and ^{125}I-rhBMP-2 (100 µl total, 20 µCi/200 µl gel) and drawn up into 1 ml syringes resulting in approximately 10 % gel dilution. ACP gel for the rat ectopic study does not contain the tracer but is diluted with MRF-00906 buffer. 200 µl injections are performed using a 22 gauge needle. The final concentration of rhBMP-2 will be 0.1 mg/ml, or 20 µg per 200 µl injection. The final concentration of ^{125}I-rhBMP-2 will be approximately 20 µCi per 200 µl injection. The ACP gel will be injected at room temperature.

In vitro release kinetics showed greatest retention of rhBMP-2 over the 2 weeks in the Hyaff-11/PEG gel followed by Hyaff-11p80 gel and Hyaff-11 gel (Fig. 1). Hyaff-11p65 gel released rhBMP-2 the fastest. Sponges and pads of Hyaff-11 and Hyaff-11p80 retained less rhBMP-2 than Hyaff-11/PEG or Hyaff-11p80 gel, but more than Hyaff-11p65. Addition of TCP to Hyaff-11 gel increased rhBMP-2 retention. The release profile in all carriers exhibited moderate to rapid burst release followed by a slow, sustained release of rhBMP-2. All Hyaff-11 and Hyaff-11p80 gel formulations retained rhBMP-2 well (> 50 % remaining after 14 days) except Hyaff-11p65.

EXAMPLE 3

RAT ECTOPIC ASSAY

Hyaff-11 based gels (200 ul/site, n = 6) with 0.1 mg/ml rhBMP-2 were injected subcutaneously (ventral thorax) or injected intramuscularly (quadriceps) in 3-4 week old male Long Evans rats. Rats were sacrificed after 2 weeks and bone formation in the explants analyzed histologically using Goldners's trichrome stain. Bone scores (0= no

bone, 5=100% bone) were assigned based on histomorphometry. Total bone (mm^3) was calculated using explant size and bone score. Radiographs of explants were also taken.

All Hyaff-11 based gels formed significant ectopic bone in the rat model (Table 1) in the presence of rhBMP-2, although differences in bone formation existed between carrier types as confirmed by radiographs and histology. Hyaff-11p65 at varying doses (0.1-1.5 mg/mL) of rhBMP-2 exhibited a dose dependent increase in bone formation (and bone score) but was inconsistent in explant size which yielded less total bone (0.1 mg/mL rhBMP-2 data shown). Hyaff-11p80 explants were large but had a lower bone score, while Hyaff-11 showed good bone score and total bone. Hyaff-11/PEG and Hyaff-11/sodium bicarbonate radiographically showed equivalent radioopacity as those of Hyaff-11 and Hyaff-11p80. Histologically, both Hyaff-11 and Hyaff-11p80 carriers showed residual remaining matrix due to their slow degradation rates, although Hyaff-11p65 completely degraded by 2 weeks. Bone formed within pores, shown by mineralizing osteoblasts as well as through a cartilage intermediate. Addition of TCP to Hyaff-11 gel with or without pore formers also showed comparable radiographic evidence of bone formation as those of other Hyaff based gels.

Table 1. Histomorphometry results of rat ectopic bone formation assay.

Group	Bone score		Total bone	(mm^3)
	SQ	IM	SQ	IM
Hyaff-11p65	2.70 (1.40)	3.88 (1.65)	79 (20)	172 (33)
Hyaff-11p80	1.83 (0.68)	1.83 (0.68)	140 (76)	314 (179)
Hyaff-11	2.50 (1.00)	3.25 (0.96)	228 (132)	219 (223)

EXAMPLE 4

IN VIVO BIODISTRIBUTION

Retention of rhBMP-2 within each carrier was analyzed in vivo using a rabbit ulna fracture model. Bilateral 0.5 mm osteotomy defects were created in the ulna of New Zealand White rabbits and 150 uL rhBMP-2/carrier injected into the defect (n=8/group). Gels were loaded with 40 uCi ^{125}I labeled rhBMP-2 and 0.67 mg/ml unlabeled rhBMP-2. Amount of radioactivity retained at the fracture site was measured by gamma scintigraphy as a function of time.

In vivo biodistribution of rhBMP-2 from Hyaff-11/PEG gel in the rabbit ulna fracture model showed better retention of rhBMP-2 than absorbable collagen sponge (ACS) and buffer carrier (MFR-842) (Fig. 2). Hyaff-11/PEG retained approximately 40 % rhBMP-2 after 7 days. Hyaff-11p65 gel showed poorer retention of rhBMP-2 than Hyaff-11/PEG gel, but displayed comparable fracture callus radiographically.

EXAMPLE 5

IN VITRO RELEASE KINETICS

rhBMP-2 was radiolabeled with ^{125}I using the Iodogen method (Pierce) and used as a tracer for 0.1 mg/ml rhBMP-2 delivered in 100 uL Hyaff-11 gel ± TCP and Hyaff-11p65 gel ± TCP (n=4). ^{125}I-rhBMP-2 loaded samples (50,000 cpm/sample) were incubated in 1 mL fetal calf serum (Hyclone) at 37°C on a shaker, and radioactivity of the carrier measured up to 14 days using a gamma counter. Fresh serum was replaced after 1, 3, 7, and 14 days.

Addition of TCP enhanced retention of rhBMP-2 over the course of 2 weeks in both Hyaff-11 and Hyaff-11p65 gels (Figure 3). Hyaff-11/TCP retained the most rhBMP-2, followed by Hyaff-11, Hyaff-11p65/TCP, and Hyaff-11p65. Hyaff-11 retained more rhBMP-2 than Hyaff-11p65 due to its hydrophobicity and insolubility. Preadsorbing rhBMP-2 on TCP increased rhBMP-2 retention in Hyaff-

11 gel, as opposed to mixing rhBMP-2 into the Hyaff-11 phase. Preadsorbing or mixing rhBMP-2 into either TCP or Hyaff-11p65 phase resulted in similar rhBMP-2 retention, both of which were greater than Hyaff-11p65 without TCP.

EXAMPLE 6

IN VIVO BIODISTRIBUTION AND EFFICACY

Retention of rhBMP-2 within Hyaff-11/TCP and Hyaff-11p65/TCP was analyzed in vivo using a rabbit ulna fracture model. Bilateral 0.5 mm osteotomy defects were created in the ulna of New Zealand White rabbits (n=3/carrier) and 150 uL carrier or buffer (0.67 mg/mL rhBMP-2) injected around the defect. 20 uCi ^{125}I-rhBMP-2 was used as a tracer. Amount of radioactivity left within each carrier at the fracture site was measured by gamma scintigraphy over the course of several weeks and in vivo rhBMP-2 retention calculated over time. Fracture repair efficacy was analyzed in these rabbits (n=8) by torsional biomechanical testing after a 4 week sacrifice to obtain maximum torque. Contralateral limbs served as surgical controls.

In vivo retention of rhBMP-2 at the rabbit ulna fracture site showed a similar pattern as that of the in vitro study (Figure 4). Hyaff-11/TCP gel (rhBMP-2 adsorbed to TCP phase first) exhibited the greatest retention (40% remaining after 4 weeks) followed by Hyaff-11p65/TCP gel (rhBMP-2 undetectable at 14 days) and buffer (undetectable at 7 days). rhBMP-2 accelerated fracture healing when delivered in Hyaff-11p65/TCP or Hyaff-11p65 gel. Maximum torque (N-m) for Hyaff-11p65/TCP and Hyaff-11p65 were significantly greater than their contralateral surgical controls (85.6% and 96.9%, respectively) but not statistically different from each other (Table 1).

Table 1. Maximum torque (N-m) of rabbit ulna defects

Carrier	rhBMP-2	Control	P value
P65	0.571	0.290	0.0001
	± 0.225	± 0.158	
P65/TCP	0.475	0.256	0.0091
	± 0.197	± 0.087	

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications are believed to be encompassed within the claims appended hereto.

CLAIMS

What is claimed is:

1. A composition for injectable delivery of osteogenic proteins comprising a pharmaceutically acceptable admixture comprising
   (a) an osteogenic protein; and
   (b) injectable hyaluronic acid derivatives.

2. The composition of claim 1 further comprising tricalcium phosphate.

3. The composition of claim 1 further comprising pore formers.

4. The composition of claim 1 wherein the osteogenic protein is selected from the group consisting of members of the BMP family.

5. The composition of claim 4 wherein the osteogenic protein is BMP-2.

6. The composition of claim 4 wherein the osteogenic protein is OP-1.

7. A composition for injectable delivery of osteogenic proteins comprising a pharmaceutically acceptable admixture comprising
   (a) BMP-2;
   (b) an injectable hyaluronic acid derivative; and
   (c) Tricalcium phosphate.

8. A composition providing an injectable matrix for osteogenic proteins comprising a hyaluronic acid derivative.

9. A method for making an injectable hyaluronic acid derivative comprising transforming insoluble or partially soluble non-woven pads or sponges of hyaluronic acid benzyl esters by hydration or solvent addition.

10. The method of claim 9 further comprising the introduction of pore formers to the solubilized carriers.

ABSTRACT OF THE DISCLOSURE

An injectable formulation is disclosed for delivery of osteogenic proteins. The formulation comprises a pharmaceutically acceptable admixture of an osteogenic protein; and formulations comprising osteogenic protein, hyaluronic acid derivatives and tricalcium phosphate are also disclosed. Methods for formulating porous injectable gels and pastes from hyaluronic acid are also disclosed.

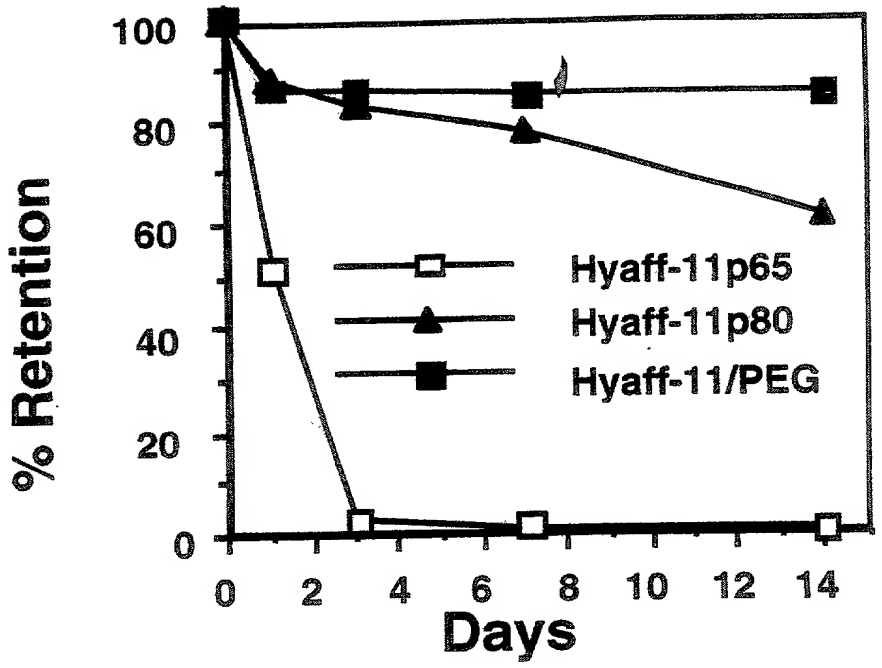

Figure 1: In vitro release kinetics of ^{125}I-rhBMP-2 in gels of Hyaff-11/PEG, Hyaff-11p80, Hyaff-11p65.

Figure 1

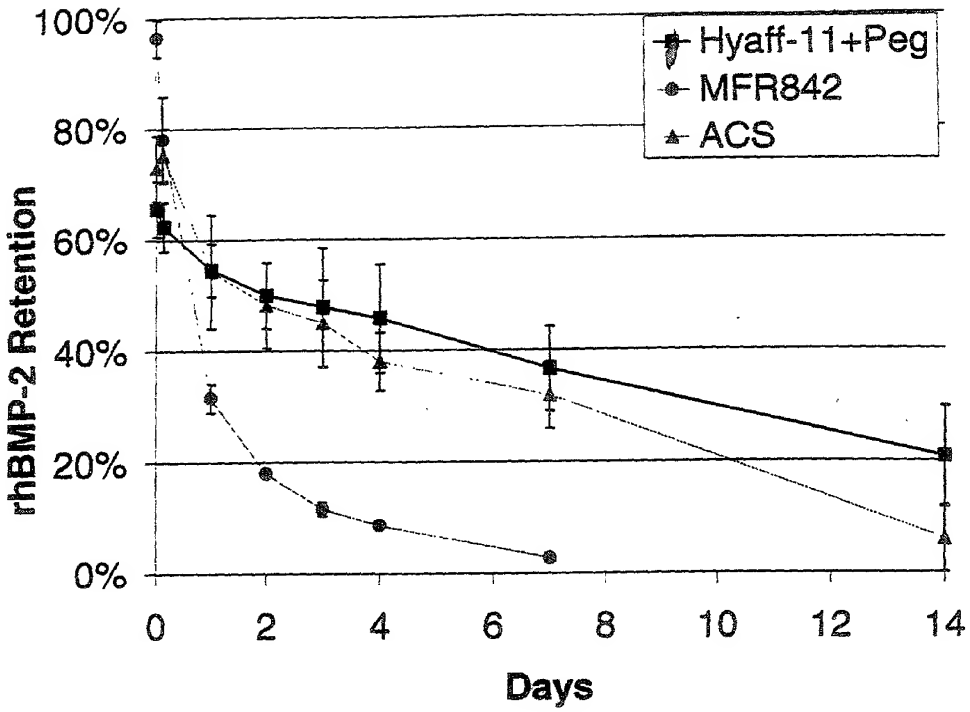

Figure 2: In vivo retention of ^{125}I-rhBMP-2 in Hyaff-11/PEG, ACS, and buffer.

Figure 2

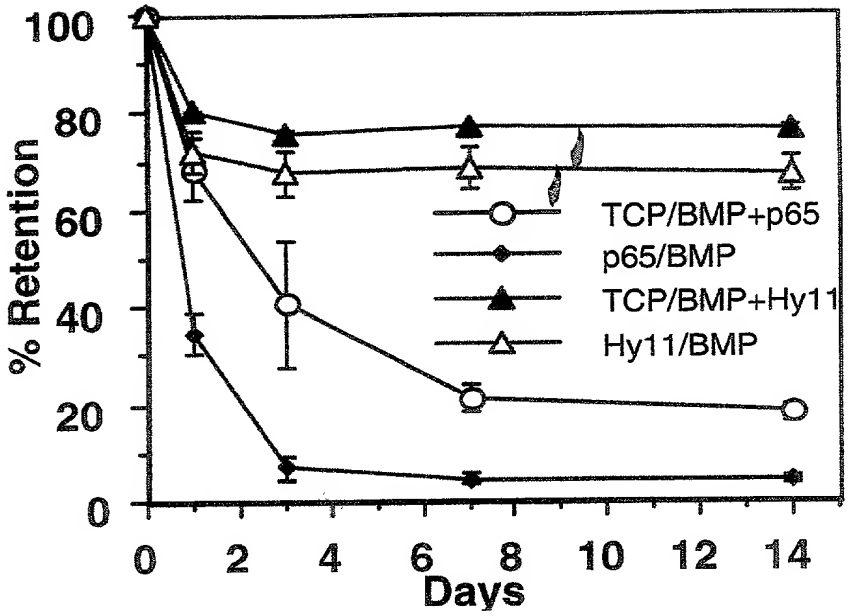

Figure 3. In vitro release kinetics of ^{125}I-rhBMP-2

Figure 3

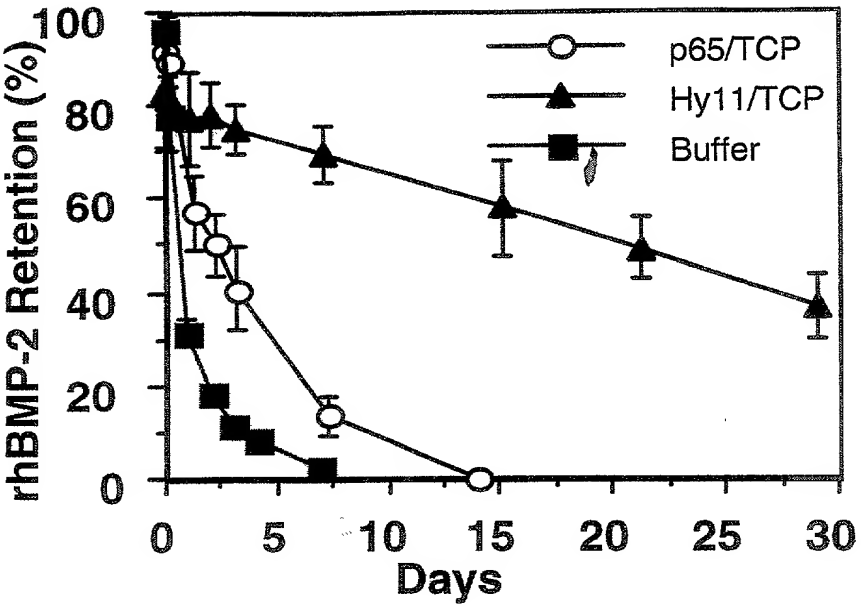

Figure 4. In vivo biodistribution of ^{125}I-rhBMP-2

Figure 4

COMBINED DECLARATION AND POWER OF ATTORNEY
(Original, Design, Supplemental, Divisional, Continuation, CIP)

As the below named inventor, I hereby declare that:

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

INJECTABLE CARRIER FORMULATIONS OF HYALURONIC ACID DERIVATIVES FOR DELIVERY OF OSTEOGENIC PROTEINS

SPECIFICATION IDENTIFICATION

the specification of which: (complete (a), (b), or (c))

(a) X is attached hereto.

(b) ☐ was filed on as
- ☐ Serial Number
- X Express Mail No. EL 564796679US, as Serial Number not yet known

(c) ☐ was described and claimed in PCT International Application No. filed on and as amended under PCT Article 19 on (if any).

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37 CFR 1.56(a).

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventors certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate of any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(d) ☐ No such applications have been filed.
(e) ☐ Such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. claimed priority, check item (e), enter the details below and make the priority claim.

Earliest Foreign Application(s), if any, filed within 12 months (6 months for Design) prior to this U.S. Application

Country	Application No.	Date of Filing (Day, Month, Year)	Priority Claimed 35 USC 119

All Foreign Application(s), if any, Filed More Than 12 Months (6 Months for Design) Prior to This U.S. Application)

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. § 119(E))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	**FILING DATE**
60/159,674	October 15, 1999
60/185,587	February 28, 2000

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)
(UNDER 35 U.S.C. 120)

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 USC 120

U.S. Applications		**Status (Check One)**		
U.S. Applications	U.S. Filing Date	Patented	Pending	Abandoned
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐

PCT Applications Designating U.S.		
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NO. ASSIGNED (if any)

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Elizabeth M. Barnhard, Reg. No. 31,088; Rebecca R. Barrett, Reg. No. 35,152; Egon E. Berg, Reg. No. 21,117; William H. Calnan, Reg. No. 29,520; Milagros A. Cepeda, Reg. No. 33,365; Steven R. Eck, Reg. No. 36,126; Bruce M. Eisen, Reg. No. 22,847; Steven H. Flynn, Reg. No. 29,639; Alan M. Gordon, Reg. No. 30,637; Barbara A. Gyure, Reg. No. 34,614; M. Kymne Hehman, Reg. No. 39,206; John W. Hogan, Jr., Reg. No. 32,703; Elizabeth A. Hurley, Reg. No. 41,859; Adley F. Mandel, Reg. No. 26,942; Gale F. Matthews, Reg. No. 32,269; Joseph M. Mazzarese, Reg. No. 32,803; Arnold S. Milowsky, Reg. No. 35,288; Daniel B. Moran, Reg. No. 41,204; Michael R. Nagy, Reg. No. 33,432; Barbara L. Renda, Reg. No. 27,626; M. Andrea Ryan, Reg. No. 28,469; George Tarnowski, Reg. No. 27,472; and Darryl L. Webster, Reg. No. 34,276 all of AMERICAN HOME PRODUCTS CORPORATION, One Campus Drive, Parsippany, New Jersey 07054.

☐ Attached as part of this declaration and power of attorney is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO:

Kay E. Brady
American Home Products Corporation
Patent Law Department
One Campus Drive
Parsippany, NJ 07054
Tel. No. (973) 683-2130

ADDRESS TELEPHONE CALLS TO:

Barbara A. Gyure
(617) 665-8653

DECLARATION

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

Full name of SOLE OR FIRST INVENTOR: **Hyun Kim**

Inventor's Signature: Date:

Country of Citizenship: South Korea
Residence : 8 Maytum Way, Middleton, Massachusetts 01949
Post Office Address: Same as above

Full name of SECOND JOINT INVENTOR: **Rebecca Li**

Inventor's Signature: Date:

Country of Citizenship: USA
Residence : 64 Putnam Street, Needham, Massachusetts 02494
Post Office Address: Same as above

Full name of THIRD JOINT INVENTOR: **Alessandra Pavesio**

Inventor's Signature: Date:

Country of Citizenship: Italy
Residence : Via Decorati al valoro civile 159, 35100, Padova, Italy
Post Office Address: Same as above

Full name of FOURTH JOINT INVENTOR: **Lanfranco Callegaro**

Inventor's Signature: Date:

Country of Citizenship: Italy
Residence : Via Monte Grappa 6, 36013-Thiene (Vicenza), Italy
Post Office Address: Same as above

Full name of FOURTH JOINT INVENTOR: **Howard Seeherman**

Inventor's Signature: Date:

Country of Citizenship: USA
Residence : 46B Trowbridge Street, Cambridge, Massachusetts 02138
Post Office Address: Same as above

Full name of FOURTH JOINT INVENTOR: **John Wozney**

Inventor's Signature: Date:

Country of Citizenship: USA
Residence : 59 Old Bolton Road, Hudson, Massachusetts 01749
Post Office Address: Same as above